16:50 - 18:20 S10 - Basic Science Hall C
Chairs: **D. Meerkin**
J. Hasin

16:50 **The transcription Factor Islet-1: A novel Gene Target for Future Cardiac Repair?**
A. Barzelay, M. Entin-Meer, S. Maysel-Auslender, E. Tzahor, E. Hochhauser, G. Keren, J. George
Tel Aviv

17:05 **Targeting HDL Quality Rather than Quantity: Providing the Mechanistic Rationale for the Pharmacogenomic Interaction Between the Haptoglobin Genotype and Vitamin E on Cardiovascular Disease in Individuals with Diabetes Mellitus**
R. Asleh, S. Blum, S. Kalet-Litman, M. Aviram, A. Levy
Haifa

17:20 **Hypoxia Inducible Factor-alpha Improves the Migratory Properties of Bone-Marrow Derived Mesenchymal cells.**
J. Semo, M. Entin-Meer, A. Barzelay, S. Maysel-Auslender, G. Keren, J. George
Tel-Aviv

17:35 **Resident Cardiac Stem Cells in the Left Atrial Appendage- an Untapped Source**
A. Korkus, Y. Helman, C. Lotan, R. Beeri
Jerusalem

17:50 **Inter-erythrocytic Cohesive Forces (RBC aggregation) are Related to Blood Velocity**
Y. Arbel, S. Banai, A. Finkelstein, N. Mashav, A. Halkin, J. George, A. Shevach, G. Keren, S. Berliner
Tel Aviv

18:05 **A Combined Cell Therapy and In Situ Tissue Engineering Approach for Myocardial Repair**
M. Habib, K. Shapira, O. Caspi, G. Arbel, A. Gepstein, D. Seliktar, L. Gepstein
Haifa

The transcription Factor Islet-1: A novel Gene Target for Future Cardiac Repair?

Aya Barzelay, Michal Entin-Meer, Sofia Maysel-Auslender, Eldad Tzahor, Edith Hochhauser, Gad Keren, Jacob George

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The LIM-homeobox transcription factor isl1 plays a crucial role during heart embryogenesis. Embryonic isl1+ precursors give rise to over two-thirds of the heart and to its subsequent lineages: cardiac muscle, smooth muscle and endothelium. Interestingly, a subset of Isl1+ progenitors remains embedded in the postnatal heart.

We have previously showed that isl1 retroviral transduction to endothelial cells improves their angiogenic properties. In this study, we investigated whether isl1 is expressed in adult mesenchymal stem cells (MSCs) physiologically, and after acute myocardial infarction (MI). Additionally, we examined whether isl1 gene transfer to MSCs could promote the cells' vasculogenic properties, and the therapeutic potential of isl1 gene delivery to the infarcted heart.

We used the transgenic mice *isl1-cre/Z/EG*, in order to detect isl1 expression in MSCs of adult mice, complemented by RT-PCR and immunostaining for isl1 detection in rats' MSCs. Four weeks after MI was induced in rats, isl1 expression was assessed in bone marrow and peripheral blood by RT-PCR and immunostaining. Isl1 was retrovirally transduced to MSCs. endothelial markers were examined by FACS and tube formation capacity was assessed on matrigel. Furthermore, intramyocardial injection of plasmid encoding isl1 to mice after ligation of the LAD has been performed.

We report for the first time, the identification of isl1+ progenitors in *adult* bone marrow. The number of isl1+ progenitors increased after *in vitro* cell culture, and also in the splenocytes after acute experimental myocardial infarction. Isl1 overexpression in MSCs promoted their differentiation towards endothelium.

These data point at the broad potential that isl1 gene therapy has in engendering cardiac repair.

Targeting HDL Quality Rather than Quantity: Providing the Mechanistic Rationale for the Pharmacogenomic Interaction Between the Haptoglobin Genotype and Vitamin E on Cardiovascular Disease in Individuals with Diabetes Mellitus

Rabea Asleh [1], Shany Blum [1], Shiri Kalet-Litman [1], Michael Aviram [2], Andrew Levy [1]

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Objective. Pharmacogenomics is a key component of personalized medicine. ICARE, a prospective placebo controlled study, recently demonstrated vitamin E could dramatically reduce CVD in individuals with Diabetes Mellitus (DM) and the Haptoglobin (Hp) 2-2 genotype (40% of DM individuals). However, due to the large number of clinical trials which failed to demonstrate benefit from vitamin E coupled with the lack of a mechanistic explanation for why vitamin E should be beneficial only in DM individuals with the Hp 2-2 genotype, enthusiasm for this pharmacogenomic paradigm has been limited. In this study we sought to provide such a mechanistic explanation based on the hypothesis that the Hp 2-2 genotype and DM interact to promote HDL oxidative modification and dysfunction.

Research Design and Methods. Clearance of ^{125}I-Hp 1 or ^{125}I-Hp 2-hemoglobin (Hb) complexes were assessed in non-DM and DM mice after injection of the complexes in the tail vein. Hb association to HDL was assessed in HDL isolated by immunoprecipitation. Oxidative modification of HDL was assessed by measuring HDL associated lipid peroxides and redox active iron. HDL function was assessed based on its ability to promote cholesterol efflux from macrophages. A crossover placebo controlled study in Hp 2-2 DM humans and in Hp 1-1 and Hp 2-2 DM mice assessed the ability of vitamin E to reduce oxidative modification of HDL and improve HDL function.

Results. In DM mice, the half-life of the Hp 2-Hb complex was dramatically increased. Immunoprecipitation studies demonstrated specific binding of Hp-Hb complex to HDL with over 25% of the injected Hp 2-Hb complex associating with HDL. Hb was found to be associated with HDL in all Hp 2-2 DM individuals and mice by western blot. Redox active iron and lipid peroxides associated with HDL were significantly increased and HDL function was remarkably impaired in Hp 2-2 DM individuals and mice. Vitamin E significantly decreased oxidative modification of HDL and improved HDL function in Hp 2-2 DM but had no effect in Hp 1-1 DM.

Conclusions. In Hp 2-2 DM individuals, there is an increased amount of Hb bound to HDL which results in an oxidative modification of HDL and HDL dysfunction. Vitamin E significantly improves the quality of HDL and may explain the exclusive CVD benefit in this cohort.

Hypoxia Inducible Factor-alpha Improves the Migratory Properties of Bone-Marrow Derived Mesenchymal cells.

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The efficacy of stem cell therapies for cardiac repair is underpinned by the need to induce appropriate migration and homing to the site of injury.
Furthermore, the benefit from self-renewal and differentiation capacities of stem cells is limited unless their migration to target tissues is appropriately orchestrated. Genetic manipulation of stem cells is a feasible approach for this purpose.
Hypoxia inducible factor (HIF) plays a pivotal role in controlling angiogenesis, erythropoiesis, vascular tone and cell motility.
Hence, we sought to investigate the effect of HIF1α and HIF2α on the migratory potential of bone-marrow derived Mesenchymal cells.
Mesenchymal cells were obtained from Wistar Rats and retrovirally modified to express stable forms of eGFP-hHIF1α and eGFP-hHIF2α. Concomitantly, total myocardial protein was extracted from adult rat heart. The migratory capacity of the transduced cells towards cardiac extract (1.7 ug/ml myocardial protein) was tested and compared to that of control mesenchymal cells transduced with eGFP only.
Interstingly, HIF2α transduced cells showed a >2-fold increase in migration capacity whereas eGFP-HIF1α or eGFP only-transduced cells showed no comparable increase.
Conclutions – HIF2α gene conferes an enhanced migratory capacity to Mesenchymal cells. This crucial functional property may enhance the therapeutic potential of stem cells in cell-based therapies for cardiac repair.

Resident Cardiac Stem Cells in the Left Atrial Appendage- an Untapped Source

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Background. In the past few years compelling evidence has accumulated suggesting that the heart may retain some regenerative potential, in reaction to pathologic stresses. Cardiac stem cells (CSC) were demonstrated to concentrate in specialized niches, rather than be uniformly distributed throughout the heart. Inferring from other organs, niches of stem cells are likely to be found mainly in crypt-like area. Thus, we hypothesized that the left atrial appendage (LAA) is a very likely place to find stem cells.

Methods. LAA tissue from mouse and rat was cut into 1- to 2 mm^2 pieces, washed and digested three times with trypsin and collagenase IV. The remaining tissue fragments were cultured as explants in complete explant medium (CEM). After three weeks, a layer of fibroblast like cells outgrew from the adherent explants. Undifferentiated cells that grew as self-adherent clusters-cardiospheres over this layer were characterized by specific stains for stem cell (c-kit) and cardiac progenitor cell (GATA4) markers. To assess clonality, single cells were seeded on matrigel and expanded in DMEM - Ham's F12(1:1) medium containing growth factors.

Results. The round undifferentiated cells grew in large numbers around the explant. These cells stained positive for c-kit and GATA4. Clones of up to 15 cells grew 4 days after separate seeding of a single cell. In one case, spontaneous differentiation occurred with spontaneous contraction.

Conclusions. We have isolated, in substantial numbers, multiplying and clonogenic cells from LAA of mice and rats. These cells exhibit a cardiac stem cell phenotype. This preliminary study suggests that the LAA may be a source of cardiac stem cells. These may be used for potential cardiac replacement therapies, and to better understand the mechanisms driving cardiomyocytes to reenter the cell cycle.

Inter-erythrocytic Cohesive Forces (RBC aggregation) are Related to Blood Velocity

Yaron Arbel [1], Shmuel Banai [2], Ariel Finkelstein [2], Noa Mashav [1], Amir Halkin [2], Jacob George [2], Ayala Shevach [1], Gad Keren [2], Shlomo Berliner [1]

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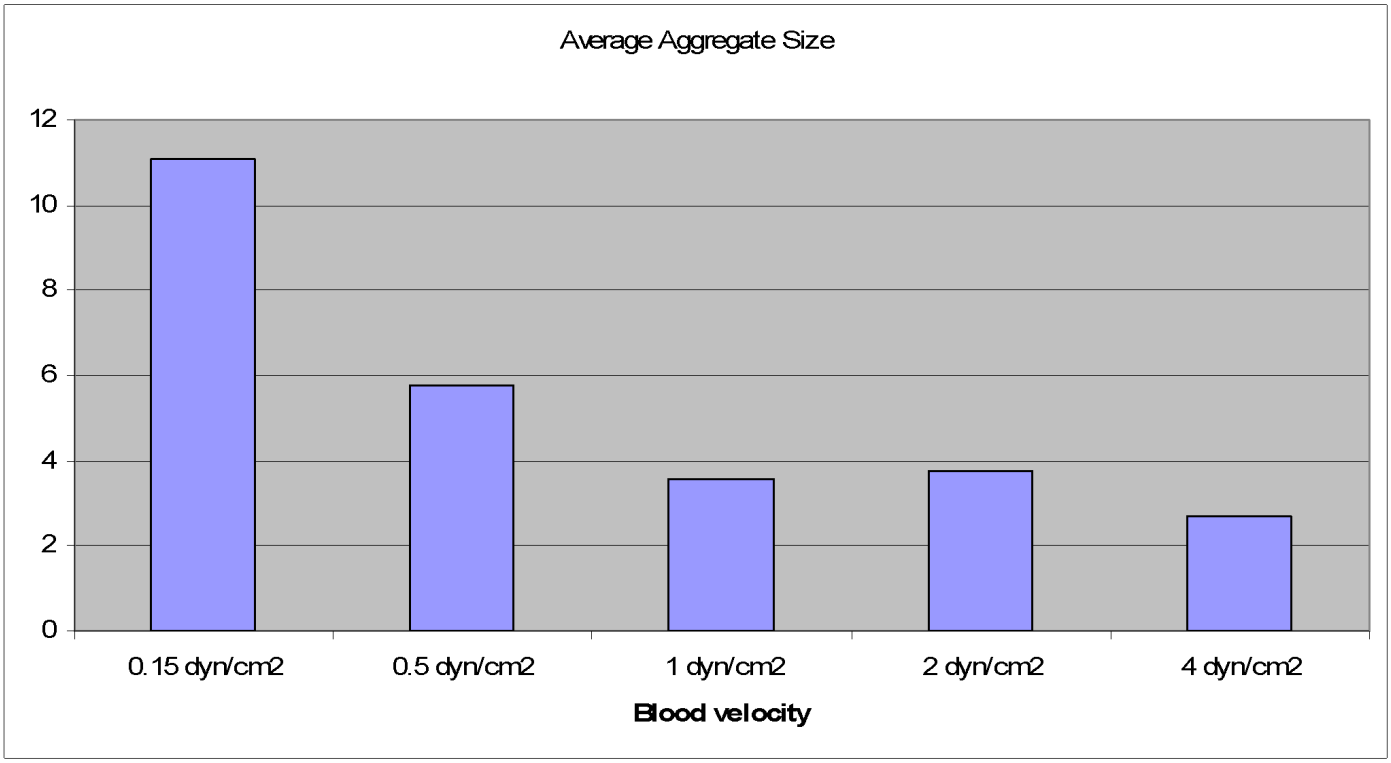
Introduction: Blood flow in the microcirculation is affected by fibrinogen and erythrocyte aggregation (EA). Since fibrinogen is constant in all blood vessels in the same individual, a change in EA will directly affect microcirculatory blood flow. The shear force of blood flow changes in different blood vessels according to their respective flow velocity. We hypothesized that EA is amplified in small blood vessels compared with large blood vessels due to different blood velocity.

Methods: Blood samples collected from 142 patients undergoing angiography were analyzed for RBC dynamics (aggregation/desegregation) using an in vitro system. RBC dynamic analysis was performed at a range of shear stress conditions (0.15, 0.5, 1.0, 2.0 and 4.0 dyn/cm^2) corresponding to different blood vessels sizes (capillaries to large arteries).

Results: EA increased significantly in low velocity blood vessels. The average aggregate size was larger in small vessels compared to large vessels (graph). The drop in aggregation with the increase in blood velocity supports the theory that EA occurs mainly in small vessels. EA was minimal in large arteries. Fibrinogen levels correlate with EA only in small blood vessels (r=0.4, p=0.001).



Conclusion: This in vitro model suggests that EA, a major determinant of blood flow in the microcirculation, occurs only in low velocity blood vessels.

A Combined Cell Therapy and In Situ Tissue Engineering Approach for Myocardial Repair

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Myocardial cell replacement and tissue engineering strategies are emerging as novel therapeutic paradigms for myocardial repair. Here we tested the hypothesis that a combined *in situ* tissue engineering and cell delivery strategy utilizing transplantable hydrogel-embedded cardiomyocytes [either neonatal rat ventricular cardiomyocytes (NRVCMs) or human embryonic stem cell-derived cardiomyocytes (hESC-CMs)] will improve functional performance in the rat recent infarction model.

Methods and results: A novel liquid biodegradable PEGylated fibrinogen was developed, which was proved to be compatible with cardiomyocyte survival and maturation *in vitro*. Photopolymerization using UV beam results in a rapid liquid to hydrogel transformation. To determine the functional consequences of the combined *in situ* cell/tissue engineering strategy, animals were randomized to injection of saline, NRVCMs alone, the biopolymer alone, or the combined delivery of the biopolymer and the NRVCMs. Histological studies demonstrated the presence of the hydrogel-embedded cardiomyocytes within the scar tissue. The biopolymer was fully absorbed one month following delivery. Echocardiographic measurements revealed typical post-infarction remodeling in the control group (saline injection) as manifested by the deterioration of ejection fraction (EF) by 28±3% (from 43±2% at post-injury baseline to 30±2% at 4 weeks). Injection of the biopolymer or NRVCMs alone prevented this remodeling process (39±2% to 39±2% and 43±3% to 43±2% respectively; $p<0.05$ when compared to controls). Co-injection of the biopolymer and NRVCMs resulted in the best functional outcome with EF improving by 22±8% from 40±4% to 48±4% ($p<0.05$ when compared to the saline or biopolymer groups and $p=0.07$ when compared to NRVCM group). Finally, initial studies also demonstrated a favorable effect following the co-injection of the biopolymer together with hESC-CMs (from a baseline value of 45±1% to 47±1%). Similar improvements in all groups were also noted for other remodeling parameters (LV diastolic area and wall motion score).

Conclusions: We describe a novel injectable in-situ-forming hydrogel that functions as an efficient cardiomyocyte carrier for both NRVCM and hESC-CMs and acts synergistically with the grafted cells to prevent the unfavorable post-infarction cardiac remodeling and improve ventricular function in rats.